

What is claimed is:

Subject

1. A reagent for performing an agglutination assay for determining the amount of an analyte in a sample, said reagent comprising:
 2. a. a mixture of microparticles, said mixture comprising first microparticles having a mean diameter and a refractive index, wherein said first microparticles are coated with a first binding partner for said analyte, and second microparticles having a mean diameter and a refractive index, wherein said second microparticles are coated with a second binding partner for said analyte, and said first microparticles having stronger light scattering properties than said second microparticles, and said first binding partner coated upon said first microparticles having a higher reactivity for said analyte than said second binding partner coated upon said second microparticles.
1. 2. The reagent of claim 1, wherein said mean diameter of said first microparticles is greater than said mean diameter of said second microparticles.
1. 3. The reagent of claim 2, wherein said refractive index of said first microparticles is greater than said refractive index of said second microparticles.
1. 4. The reagent of claim 3, wherein a ratio of the mean diameter of said first microparticles to the mean diameter of said second microparticles ranges from about 1.5 to about 4.0.

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1 5. The reagent of claim 4, wherein the ratio of the mean diameter of said first
2 microparticles to the mean diameter of said second microparticles ranges from
3 about 1.7 to about 3.2.

1 6. The reagent of claim 3, wherein a ratio of the concentration of said first
2 microparticles and the concentration of said second microparticles in said mixture
3 ranges from about 0.01 to about 5.0.

1 7. The reagent of claim 6, wherein a ratio of the concentration of said first
2 microparticles and the concentration of said second microparticles in said mixture
3 ranges from about 0.05 to about 2.0.

1 8. The reagent of claim 1, wherein a ratio of the detection limits of an assay
2 performed with said first microparticles and the detection limits of an assay
3 performed with said second microparticles ranges from about 0.01 to about 5.0.

1 9. The reagent of claim 1, wherein said analyte is a nucleic acid and said first and
2 second binding partners are oligonucleotide capture probes.

1 10. The reagent of claim 1, wherein said analyte is antigenic and said first and second
2 binding partners are immunological binding partners.

1 11. The reagent of claim 10, wherein a ratio of the dissociation constants of said first
2 and second binding partners for said first and second microparticles is from about
3 0.01 to about 5.

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12. The reagent of claim 10, wherein said first and second binding partners are monoclonal antibodies or fragments thereof.

1 13. The reagent of claim 12, wherein said analyte comprises non-repetitive epitopes,
2 said first microparticles are coated with at least two sets of first binding partners
3 reactive for different epitopes on said analyte, and said second microparticles are
4 coated with at least two sets of second binding partners reactive for different
5 epitopes on said analyte.

1 14. The reagent of claim 12, wherein said analyte comprises non-repetitive epitopes,
2 and said first microparticles comprise a first portion and a second portion, wherein
3 said first portion is coated with a first binding partner portion reactive with said
4 analyte and said second portion is coated with a second binding partner portion
5 reactive with said analyte; and said second microparticles comprise a first portion
6 and a second portion, wherein said first portion is coated with a third binding
7 partner portion reactive with said analyte and said second portion is coated with a
8 fourth binding partner portion reactive with said analyte, wherein said third and
9 fourth binding partner portions coated upon said second microparticles have lower
10 reactivities for said analyte than said first and second binding partner portions
11 coated upon said first microparticles, and said first, second, third, and fourth
12 binding partner portions are directed to different epitopes of said analyte
13 respectively.

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1 15. The reagent of claim 13, wherein the mean diameter of said first microparticles is greater than the mean diameter of said second microparticles.

1 16. The reagent of claim 15, wherein the refractive index of said first microparticles is greater than the refractive index of said second microparticles.

1 17. The reagent of claim 14, wherein the mean diameter of said first microparticles is greater than the mean diameter of said second microparticles.

1 18. The reagent of claim 1 wherein said assay for determining the amount of analyte in a sample is an agglutination assay.

1 19. The reagent of claim 1, wherein the composition of said first and second microparticles is selected from the group consisting of inorganic, organic and polymer materials suitable for microparticle enhanced light scattering assays.

1 20. The reagent of claim 1, wherein the composition of said first and second microparticles is selected from the group consisting of selenium, carbon, gold, a nitride of carbon, a nitride of silicon, a nitride of germanium, an oxide of iron, an oxide of titanium, an oxide of silicon, an epoxy resin, polyvinyl chloride, polyvinylidene chloride, polyalpha-naphthylmethacrylate, polvinylnaphthalene, polystyrene and a copolymer thereof.

1 21. A method of preparing a microparticle reagent which comprises mixing first microparticles of 30-600 nm in diameter having a refractive index wherein said first microparticles are coated with a first binding partner reactive to an analyte, and

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4 second microparticles of 30-600 nm in diameter having a refractive index wherein
5 said second microparticles are coated with a second binding partner reactive for
6 said analyte and said first microparticles have stronger light scattering properties
7 than said second microparticles, and said first binding partner coated upon said
8 first microparticles has a higher reactivity for said analyte than said second binding
9 partner coated upon said second microparticles.